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## NO DRAWINGS.

PATENT SPECIFICATION



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Application made in United States of America (No. 532,045) on Feb. 18, 1966.

(Patent of Addition to No. 1,037,408 dated May 2, 1963). Complete Specification Published: Oct. 11, 1967.

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## COMPLETE SPECIFICATION.

## Dough and Baked Products Prepared Therefrom.

We, R. T. VANDERBILT COMPANY, INC., a corporation duly organized and existing under the laws of the State of New York, and having an office at 230 Park Avenue, New York, New York, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and

by the following statement:—
This application is an improvement in or modification of the invention which is the subject of our copending Application No. 17416/63 (Serial No. 1,037,408) which is concerned with the production of improved baked products and more particularly with the production of bread, rolls and sweet goods that remain soft longer.

As explained in the specification accompanying that application, there is a con-sumer demand for baked goods, especially bread, which will keep fresh for longer periods.

Various expedients have been practised including the incorporation in the dough of additives such as monoglycerides. Although these materials provide improved results, they may give undesirable changes in the cell structure of the bread. This is often associated with the use of monoglycerides made from fats having a high iodine number. The effect is more noticeable as the products containing monoglycerides are permitted to age.

As described in the specification of the parent application, it has been found that the use of certain lipase preparations in a dough mixture will significantly retard the tendency of the baked goods made from the dough to become stale. At the same time, the baked goods made in this manner exhibit none of the disadvantages usually en-[Price 4s. 6d.]

countered when monoglycerides are used as staling retarding additives. The lipase pre-paration is added to the dough along with the flour, and water, and any other ingredients such as shortening and yeast. dough is mixed until smooth and is thereafter handled in the conventional manner.

Lipases are enzymes which accelerate decomposition of triglyceride fats. composition products are mainly diglycerides, monoglycerides, fatty acids and glycerine, the relative amounts and proportions of each depending upon the characteristics of the lipase preparation and the length of time over which it is permitted to act.

The lipases are individualistic in their behaviour according to their source, conditions of preparation and use. Some produce an abundance of one end product at the expense of another. Some lipases are more effective in acid medium while others are more effective with fatty acids of a particular type and are relatively or completely ineffective with others. Some may be crystallized as pure materials; others, as extensive trials have shown, are not pure materials but are mixtures of active components which are ineffective when separated. Most commercial lipase preparations contain, in addition to lipase, other types of enzymes in appreciable quantities.

It has now been found that the lipase preparation hereinafter described is particularly effective in retarding staling of baked goods when incorporated in the dough mixture before baking. This lipase preparation employed in this invention is obtained by cultivation of the microorganism Candida cylindracca ATCC No. 14.830 in a suitable medium at a temperature of from 20-35°C. The product and the method of obtaining

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Paient Specification No. 976,415.

Dough in accordance with the invention comprises flour and the lipase preparation

herein specified. The lipase preparation is of benefit both in doughs containing added far as shortening and in those doughs which do not use an added shortening increment. 10 Flour ordinarily contains about 1.5% of lipid materials, most of which can be extracted by solvents such as acc-tone, or ether. It has been noted that once flour has been made into dough by the 15 addition of water and mixing, the lipids apparently become bound by the protein so that only about 0.15% of the lipids can be extracted by ether or acctone.

While the exact mechanism is not known, 20 it is thought that the lipase splits the natural flour lipids to form monoglycerides, and that these monoglycerides are, to some ex-tent, preferentially bound by the flour pro-tein displacing the linid component. The tein, displacing the lipid component. increased softness therefore is thought to be the added effect of the monoglyceride on the starch and the possible lipo-protein modifying action resulting from an increase in solvent-extractable lipids.

The potency of a lipase is commonly expressed in terms of free fatty acids produced under standardized conditions. This, of course, is an index of its ability to split a triglyceride but is not necessarily a measure of its ability to produce monoglycer-ide. The value of lipase as an anti-staling agent in bread is more closely related to its potency expressed in terms of monogly-

it are described and claimed in British ceride production than expressed in terms of ires fatty acid production. Accordingly, its effectiveness may be gauged more accurately by the amount of extractable monophyceride which is in the bread pro-

Effective amounts of lipase should produce en increase in extractable monoglycerides of at least 0.5 ounce per 100 pounds of flour. The maximum amount of lipase required depends on the reduction in firm-ness and staling rate desired. In yeast raised sweet goods, for instance, the increase in extractable monoglycerides may be as much as 32 ounces per 100 pounds of flour.

Some monoglyceride is produced in bread during baking due to splitting of fats at elevated temperatures in the presence of water and salts. This monoglyceride may amount to 4 ounces per 100 pounds of flour. There is some evidence that this monoglyceride is formed too late in the bread making process to form anti-firming agents in bread. This monoglyceride is perhaps not "available" at a critical stage.

Whatever may be the effect of monogly-ceride produced in the absence of lipase, it is clear that when lipase is added more monoglyceride is produced, and the desirable anti-firming effects are noticed with the first increase in extractable monoglyceride.

The invention will now be illustrated in the following non-limitative Example.

EXAMPLE

White pan bread was prepared according to the following recipes:

SPONGE

70 lbs. Hard Wheat Flour

51 lbs. Water

11 lbs. Yeast (Dry) 1 lb. Yeast Food

75

80

30 lbs. Hard Wheat Flour

15 lbs. Water

2 lbs. Salt

5 lbs. Sugar

4 lbs. Dry Milk

4 lbs. Shortening

1 lb. Lipase Preparation

The sponge was prepared by dissolving 85 the yeast in a portion of the water at 110°F. and the solution was added to the mixer along with flour, yeast food and the bal-ance of the water. The materials were mixed just enough to make a homogeneous mass, dumped into a trough and fermented for 3 hours at 78°F.

The fermented sponge was returned to the mixer, all of the dough ingredients were added and the batch was mixed until smooth. The dough was allowed to stand about 15 minutes, divided, rounded and allowed to stand again. It was then moulded, panned, proofed at 95°F, to the top of the pans, and baked at 420°F, until uniformly brown, i.e. about 30 minutes, with steam in 100 the oven. The loaves were cooled slowly to room temperature and wrapped in moisture proof paper.

Representative samples were taken immediately after baking and analysed for 105 extractable monoglyceride content. Firmness of the resulting bread was measured objectively after three days using a Baker Compressimeter. The results obtained are shown in the following table:





			Lipase	Control (lipase conitted)
Firmness after 3 days as percent of control		•••	73	100
Percent reduction with respect to control Extractable monoglyceride content of bread	•••	•••	27	O
as % by weight of flour	. ***	***	.74	.11

The lipase preparation used was obtained by cultivation of the microorganism 10 Candida cylindracea ATCC No. 14,830 as described in British Specification No. 976,415. The activity of the lipase so prepared is subject to variations depending, for example, on the age of the culture, the growth medium and the precise condition of fermentation. In this Example the lipase used had an activity of 21,000 u/g.

The enzyme unit is defined as the amount of enzyme required to liberate 1 mole of 20 fatty acid per minute under the following

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Into a glass-stoppered Erlenmyer flask of 50 ml. capacity are placed 5 ml. of an olive oil emulsion and 0.4 ml. of a 0.1M phosphate buffer having a pH of 7.0. The olive oil emulsion is prepared by blending 22.9 gm. of olive oil and 75 ml. of a 2% polyvinyl alcohol solution in a high-speed homogenizer. The contents of the flask are mixed well and heated to 37°C. on a water bath.

To this solution 1 ml. of a sample solution containing a known amount of a lipase preparation is added to the flask. The flask is shaken 15 seconds to disperse the enzyme, and then incubated at 37°C. for 20 minutes. After exactly 20 minutes, 20 ml. of a 50:50 acetone: ethanol solution and 5 drops of phenolphthaleine indicator are added. The contents of the flask are then titrated with 0.05N NaOH.

A blank is prepared by following the foregoing procedure except that the actione-ethanol solution is added before the enzyme preparation has been added and the contents of the flask incubated.

The enzyme activity is calculated in units

per gram as follows:

u/g = (ml. of NaOH for sample) - (ml. of NaOH for blank) × 2.5.

gms of enzyme

For best results the amount of enzyme used in the foregoing test should be sufficient to yield a titration value of 1.0 - 2.0 ml. of 0.05N NaOH.

WHAT WE CLAIM IS:-

1. A dough comprising flour and a lipase preparation as hereinbefore specified.

2. A doogh according to claim 1 which

also contains shortening.

A dough according to claim 1 or claim 2, in which the lipase preparation is present in an amount sufficient to increase the extractable monoglyceride content of the dough by at least 0.5 ounce per 100 pounds of flour.

4. Baked products prepared from a

dough as claimed in any of the preceding 65

5. A method of preparing baked products employing a dough according to any of claims 1 to 3.

6. The method of preparing bread substantially as described with reference to the Example.

Bread whenever prepared by the method as claimed in claim 5 or claim 6.

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